

Appl. No. 09/714,040
Amendment dated April 30, 2007
Reply to Office Action of November 30, 2006

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Amendments to the Specification:

Please amend the paragraph beginning at page 30, line 17 as follows:

Formation of $F(ab')_2$ molecules requires chance encounters of Fab' hinge cysteinyl thiols to form disulfide bonds without the assistance of extensive interactions between C_H3 domains possible in the case of intact antibodies. Thus high level expression of Fab' in the periplasmic space of *E. coli* was anticipated to drive formation of $F(ab')_2$ *in vivo*. In fact < 10% of the Fab' molecule having the CPC CPPC terminus (isolated from either media or cell paste) were recovered as the bivalent form as judged by SDS-PAGE analysis after protein A purification. High resolution mass spectrometry and other studies suggested that substantial formation of an intramolecular disulfide bond between the two hinge cysteine residues had occurred. This possibility was precluded by the construction of an additional Fab' variant with a single hinge cysteine residue having the C-terminal sequence, Cys Ala Ala. Negligible quantities of $F(ab')_2$ are formed when this Fab' variant is secreted from *E. coli* and DTNB analysis does not detect free thiol.